Oxidative Stress and Inflammation in Atherosclerosis: A Brief Review

Shahida Perween[1], Moinuddin[1], Shagufta Moin[1], Abul Faiz Faizy[1*],
[1] Department of biochemistry, Faculty of Medicine, Jawaharlal Nehru Medical Collage, Aligarh Muslim University, Aligarh, U.P.-202002
Department of biochemistry, Faculty of Medicine, Jawaharlal Nehru Medical Collage, Aligarh Muslim University, Aligarh, U.P.-202002

Abstract: Atherosclerosis is the hardening of the arteries due to plaque formation. Oxidative stress and inflammation are involved in the initiation and propagation of atherosclerosis, mainly through oxidative modification of low density lipoprotein. Reactive oxygen or nitrogen species are released by NAD(P)H oxidase, nitric oxide synthase, xanthine oxidase, lypoxygenase or myeloperoxidase. Body has antioxidants defense system (enzymatic and non enzymatic) for the prevention of accumulation of free radicals. Imbalance between oxidants and antioxidants results in oxidative modification in the arterial wall leading to atherosclerosis. This review focuses on the participation of reactive oxygen species (ROS), reactive nitrogen species (RNS) and inflammation in atherosclerosis.

Key words: Oxidative stress; Reactive oxygen species; Reactive nitrogen species; Antioxidant enzymes; Atherosclerosis

Introduction

Marchand introduced the term "atherosclerosis", describing vessel stiffening and fatty acid degeneration [3, 10]. An increased oxidative stress may result Inflammation, various animal studies suggest the role of oxidative stress in atherosclerosis, particularly through oxidative modification of low density lipoprotein (LDL) and the uptake of oxidized LDL by macrophages is easier relative to non-oxidized lipoproteins [60]. Atherosclerosis is characterized by the flooding of cholesterol deposits in macrophages in arteries. As a result proliferation of the few cell types occurs in the arterial wall, thereby affecting the vessel lumen and hindering the blood flow. These resulting lesions are called "fatty streaks", (can usually be found in the aorta in the first decade of life, in the coronary arteries in the second decade, and in the cerebral arteries in the third or fourth decades). Clinically, these streaks become significant, under they form more advanced lesions which characterized by the addition of lipid rich necrotic debris and smooth muscle cells [40].Within the arterial wall, oxidative stress and inflammation are inter connected and mutually support to accelerate atheroma formation [60]. In atheroma, oxidized LDL and its components activate the innate immune system by ligating Toll-like receptors. These interactions spark an intracellular signaling cascade leading to increased expression of a range of proinflammatory molecules, including ROS, reactive nitrogen species, chemokines, cytokines, eicosanoids, proteases and costimulatory molecules [22]. Oxidized phospholipids are important biomarkers that exert mixed effects on atherosclerosis, including promotion of monocyte adhesion to endothelial cells; increased production of proinflammatory cytokines, chemokines, and growth factors; suppression of inflammation in leukocytes; and stimulation of smooth muscle cell proliferation (Figure 1) [36].

Oxidative Stress and inflammation

Imbalance between generation of ROS and body antioxidant defense leads to oxidative stress. In stress conditions, increase the levels of ROS produce to a variety of reactions because of their high reactivity and also play an important role in necrosis, apoptosis and cell damage, via oxidation of lipids, proteins and DNA, [14] and also causes endothelial cell damage, intrusion, and proliferation of inflammatory cells [29]. Mostly ROS are generated from mitochondria and membrane oxidases [62]. Oxidative stress induced the endothelial dysfunction is involved at various stages of the formation of atherosclerotic plaque. Bioavailability of NO is reduces by oxidative modification. Moreover peroxynitrite produced modification results in the formation of various harmful factors. Atherothrombosis is particularly caused by oxidative stress; therefore efforts have been made for the identification of biomarkers for the detection of oxidative stress which will allow the management of risk factors [56, 23, 17]. These free radicals play a crucial role in various conditions such as atherosclerosis, congestive heart failure, ischemic heart disease, cardiomyopathy, arrhythmias, ischemia reperfusion, diabetes and cancer [9, 24, 33, 69]. In cardiovascular disease the source oxidative molecules are uncoupled nitric oxide

synthases [24, 65, 67] xanthine oxidase [51] cyclooxygenase [26] lipoxigenase [27] cytochrome P450 [15], NAD(P)H oxidase [18] and mitochondrial respiration [4, 55]. These are considered as the potent source of ROS in all type of vasculature. ROS include peroxynitrite ($ONOO^-$), nitric oxide (NO) superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\cdot}OH$) [figure 2]. Low concentrations of ROS are produced under physiological conditions. These reactive species act as signaling molecules which are induced in the regulation of vascular smooth muscle cell and its contraction and relaxation [63, 54, 68]. Reduction of laminar where stress may result in decreased formation of endothelium-derived nitric oxide. Anti-inflammatory properties of NO are important for limit the expression of VCAM-1 [12]. In addition disturbed blood flow results in the inhibition of protective mechanism of the body and also promotes the formation of certain adhesion molecules such as ICAM-1 [48]. Formation of proteoglycans by smooth muscle cells (SMCs) is in increased by increased wall stresses. These proteoglycans can bind and maintain lipoprotein molecules truly promoting their oxidative modification and hence inflammation is increased at sites of lesion formation [35]. T cells also result in the increased formation of inflammatory cytokines such as γ-interferon (tumor necrosis factor [TNF]-β) and lymphotoxin that can stimulate macrophages SMCs and vascular endothelial cells [21]. Initiation and evolution of atheroma are the result of Inflammation, which also promote acute thrombotic complications of atheroma. Disruption of the atherosclerotic plaque may cause fatal acute myocardial infarction. Macrophages which areabundant in atheroma can produce certain proteolytic enzymes which are capable of mortifying the collagen that gives strength to the plaque's protective fibrous cap, become thin, and can be easily ruptured. In the plaque, γ-Interferon produced, lymphocytes T cell can arrest the synthesis of collagen by SMCs, thus limiting its ability to renew the collagens which support the formation of plaque (Figure 1) [37, 38].

Reactive oxygen and nitrogen species (ROS & RNS) generation and their effects on signaling systems in atherosclerosis (Fig 2)

Role of enzyme systems in the Atherosclerosis

NAD(P)H oxidase, nitric oxide synthase, xanthine oxidase, lipoxygenase and myeloperoxdase enzymes are the major producer of oxidant in endothelial cells. Due to imbalance in these enzymes vascular function gets is impaired and can lead to atherosclerosis.

NAD(P)H Oxidase

One of the major source of free radicals (O_2^-, H_2O_2 & NO) is NAD(P)H oxidase which is in myocytes, cardiac tissue and vascular cells. One of the various membrane-bound enzymes are NAD(P)H oxidases (cardiovascular system) with oxidation-reduction reaction and uses NADH/ NADPH as reducing agent (Equation 1).

$$NAD(P)H\ 2O_2 \rightarrow\ NAD(P)^+ +\ H^+ + 2O_2\text{-} \qquad (1)$$

In vascular tissue and cardiac cells NADPH oxidase is found to be the major oxidase [37] in the production of ROS [46,49,53], as compared to the xanthine oxidase, arachidonic acid, and mitochondrial oxidases. ROS produced due to NADPH oxidase activity, deactivate nitric oxide and stimulate ROS signaling pathways and leads to various disease progressions. Nox2 and Nox4 activity found to be increased in atherosclerosis of coronary arteries in humans [19, 57].

Nitric oxide synthase (NOs)

NO synthases (NOs) catalyze the oxidation of L-arginine to L-citrulline to produce Nitric oxide (NO) [44, 64]. One of the critical steps of atherogenesis is endothelial dysfunction, as a result of disturbance in endothelium dependant relaxation (EDR) leading to decreased eNOS activity. Among various phenomena responsible for damaged EDR, the most important is the increment of NO breakdown through increased superoxide in atherosclerotic walls. eNOS enzymatic activity is repressed by various phenomena linked with atherosclerosis and hyperlipidemia. Transduction of signal from receptor activation to eNOS activation is inhibited by oxidized low-density lipoprotein (oxLDL) and lysophosphatidylcholine [25, 30, 45]. The important steps of atherogenesis that is leukocyte endothelial adhesion, vascular smooth muscle migration and proliferation, and platelet aggregation is inhibited by NO from eNOS. Their exist controversy and exact mechanisms is still not known. Atherosclerotic lesion which is found in animal models is damaged by long lasting treatment of L-arginine, which is a substrate for NOS (Fig 3) [2, 8].

Xanthine oxidase (XO)

The surface of endothelial cells is bound by glycosaminoglycans [28] and releases Xanthine oxidase into plasma through heparin bolus injection [1, 34, 58]. Conversion of hypoxanthine to xanthine and xanthine to urate is catalyzed by Xanthine oxidase. As the dehydrogenase (XDH) enzyme is activated under physiological conditions which is converted to superoxide producing XO to promote various stimuli such as inflammation

and hypoxia. Xanthine oxidase (XO) activity increased in hyperlipidemia which was performed by several human and animal studies [5, 31] but the progression of atherosclerosis is not clear now (Fig 4) [61].

Lipoxygenase

Lipoxygenases are defined as non-heme iron-containing enzymes that catalyze the molecular oxygen into polyunsaturated fatty acids with a 1, 4-cis-pentadiene motif [6]. Low-density lipoproteins (LDL) play a major role in atherosclerosis and coronary artery disease (CAD). 12-lipoxygenase was found human atherosclerotic plaques which is important for the development of atherosclerotic disease [59]. Arachidonic acid can be broken down into leukotrienes and lipoxin in the presence of 5-lipoxygenase (5LO) which is produced by macrophages (Equation 2, 3). These molecules causes increased vascular permeability, chronic inflammation and vasoconstriction and thereby the progression of atherosclerotic plaques and also hypothesized that the 5LO have early studies in genetic and knockout mouse which play a major role in the formation of plaque growth [42].

$$Fatty\ acid + O_2 \xrightarrow{Lipoxygenase} Fatty\ acid\ hydroperoxide \quad (2)$$

$$Eg.\left(Arachidonic\ acid \xrightarrow{Lipoxygenase} Leukotrienes + HO^{\cdot}\right)$$

$$Fatty\ acid\ hydroperoxide \xrightarrow{Isomerase} 1{,}5\ epoxide + OH^{\cdot} \quad (3)$$

Myeloperoxidase (MPO)

Myeloperoxidase, released from activated neutrophils and monocytes catalyzes the formation of hypochloric acid from hydrogen peroxide mediated halogenation reaction. Lysine residue of the ApoB-100 moiety of low-density lipoprotein (LDL) is oxidized by hypochloric acid. As a result oxidized LDL is generated which appears to be foreign by the cells thereby activating immune system. Blood MPO level can be used for predicting the development of atherosclerosis, as revealed by some studies (fig 5) [7].

Antioxidant Defense

Free radicals, produced by oxidation reactions can start chain reactions leading to cell damage. An Antioxidant is a molecule which can slow down and prevent the oxidation of other molecules [13]. Antioxidant defense systems operates at various steps such as blocking the free radical production, scavenging or converting the oxidants to less toxic compounds, blocking the chain propagation of the secondary oxidants, blocking the secondary production of inflammatory mediators or toxic metabolites, repairing the free radical induced molecular injury or by increasing the antioxidant defense mechanism of the target. Body is protected from oxidative stress by simultaneous operation of antioxidant defense mechanism. Antioxidant defense system consists of enzymatic and non-enzymatic antioxidants [20].

Enzymatic Antioxidants

Superoxide dismutase (SOD)

SOD catalyzes the conversion of superoxide radical to hydrogen peroxide as follows

$$O_2^{\cdot-} + O_2^{\cdot-} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2 \qquad (4)$$

It prevents the oxidative damage by intercepting free radicals before their harmful damaging action on intracellular targets [11, 41]. In humans, there are three forms of SOD: cytosolic (Cu, Zn-SOD), mitochondrial (Mn-SOD) and extracellular (EC-SOD) [65].

Catalase

Catalase and glutathione peroxidase produces protection against H_2O_2 by converting it to H_2O and O_2 thereby protecting cells from oxidative stress.

$$2H_2O_2 \xrightarrow{Catalase} 2H_2O + O_2 \qquad (5)$$

Oxidation of LDL may be related to the activities of these antioxidants. Decreased activity of catalase in erythrocytes of patients with Coronary Artery Disease (CAD) has been reported [32].

Glutathione system

Glutathione is a natural defense system responsible for the regulation of the intracellular redox state and scavenging of reactive oxygen species. It comprises of glutathione (GSH), glutathione peroxidase (GPx) and

glutathione reductase (GR). GPx is an enzyme that catalyzes the reduction of H_2O_2 to H_2O by utilizing glutathione as a co-substrate:

$$H_2O_2 + 2GSH \xrightarrow{GP_X} GSSG + 2H_2O \quad (6)$$

Glutathione disulfide (GSSG) is then reduced back to GSH by GSH reductase:

$$GSSG + NADP + H^+ \xrightarrow{glutatione\ reductase} 2GSH + NADP^+ \quad (7)$$

The efficiency of cells in managing oxidative stress depends on its activity to regenerate GSH [50].

Non-enzymatic antioxidants

Ascorbic acid (Vitamin C)

Ascorbic acid is a natural antioxidant, found in both plants and animals. It cannot be synthesized in humans and must be obtained from diet. It can reduce and neutralize reactive oxygen species such as H_2O_2. However in cells, it is maintained in its reduced states by reaction with glutathione [39]. The antioxidant mechanism in the human body is

$$2Fe^{2+} + 2H_2O_2 \rightarrow 2Fe^{3+} + 2OH^{\cdot} + 2OH^- \quad (8)$$

$$2Fe^{3+} + Ascorbate \rightarrow 2Fe^{2+} + Dehydroscorbate \quad (9)$$

Tocopherols (Vitamin E)

Vitamin E is the fat soluble compounds with unique antioxidant properties. Vitamin E has been hypothesized to prevent oxidative modification of LDL. Studies have shown beneficial effect of vitamin E in the reduction of relative risk of CVD [16]. It has been found to inhibit the production of chemokines (such as IL-8 & MCP-1) by endothelial cells. It has also been found to inhibit the attraction of monocytes to inflammatory sites in the arterial wall [66].

Mechanism of tocopherol in human body

$$Unsaturated\ fatty\ acid\ (ROOH) + HO^{\cdot} \rightarrow Fatty\ acyl\ radical\ (RO^{\cdot}) + H_2O \quad (10)$$

$$RO^{\cdot} + O_2 \rightarrow Peroxy\ radical\ (ROO^{\cdot}) \quad (11)$$

$$ROO^{\cdot} + ROOH \rightarrow ROOR + H_2O \quad (12)$$

In normal condition the peroxy radicals are trapped by α tocopherol molecules oxidized and give tocopheroxy radical.

$$RCOO^{\cdot} + \alpha\ tocopherol \rightarrow ROOH + \alpha\ tocopheroxy\ radical \quad (13)$$

β- carotene

β-carotene is antioxidant in nature and it can enhance the proliferation of lymphocytes. It has been found to reduce the susceptibility of oxidative modification LDL by enriching it. β- carotene has been reported to quench oxygen free radicals [66]. It may also react with peroxyl radical at low oxygen tensions. Vitamin E also reacts with peroxyl radicals but at higher oxygen tensions.

$$\beta carotene\ (CAR) + Peroxy\ radical\ (ROO^{\cdot}) \rightarrow ROO - \beta\ carotene\ (ROO - CAR) \quad (14)$$

$$ROO - CAR + ROO^{\cdot} \rightarrow ROO - CAR - OOR \quad (15)$$

Conclusion

In this paper, we have discussed the role of oxidative stress in atherosclerosis. In the arterial wall, synergize between oxidative stress and inflammation increase the risk for atherosclerotic plaque formation which may lead to cerebral stroke or myocardial infarction. Over production of free radicals may have damaging effects on the human body. Enzymatic and Non-enzymatic antioxidants protect the body from these effects. Further studies are needed to be conducted in detail by which antioxidant prevents the harmful effects of oxidative stress and inflammation.

References

1. Adachi T et al: Binding of human xanthine oxidase to sulphated glycosaminoglycans on the endothelial-cell surface. Biochem J. 1993, 289 (2): 523-527.
2. Aji W et al: L-arginine prevents xanthoma development and inhibits atherosclerosis in LDL receptor knockout mice. Circulation 1997, 95: 430–437.
3. Aschoff L. Introduction in arteriosclerosis: A survey of problem. Cowdry EV (ed) Macmillan New York, 1933.
4. Ballinger SW et al: Mitochondrial integrity and function in atherogenesis. Circulation 2002, 106: 544-549.
5. Berry CE et al: Xanthine oxidoreductase and cardiovascular disease: molecular mechanisms and pathophysiological implications. J. Physiol.2004, 555: 589–606.
6. Brash AR: Lipoxygenases occurrence, functions, catalysis, and acquisition of substrate. J Biol Chem. 1999, 274: 23679 –23682.
7. Brennan ML et al: Prognostic value of myeloperoxidase in Patients with Chest Pain. The New England Journal of Medicine 2003, 349:1595-1604.
8. Candipan RC et al: Regression or progression, dependency on vascular nitric oxide. Arterioscler ThrombVasc Biol. 1996, 16: 44–50.
9. Cosentino F et al: Role of superoxide anions in the mediation of endothelium dependent contractions. Hypertension 1994, 23: 229-235.
10. Crowther MA: Pathogenesis of atherosclerosis. Hematology. Am. Soc. Hematol. Educ. Program. 2005, 436-441.
11. Curtin JF et al: Regulation and measurement of oxidative stress in apoptosis. J Immunol Methods 2002, 265(1–2):49–72.
12. De Caterina R et al: Nitric oxide decreases cytokine induced endothelial activation: nitric oxide selectively reduces endothelial expression of adhesion molecules and proinflammatory cytokines.J Clin Invest. 1995, 96:60–68.
13. Duarte TL et al: When is an antioxidant not an antioxidant? A review of novel actions and reactions of vitamin C. Free Radic Res 2005, 39 (7): 671-86.

14. Elahi MM et al: Oxidative stress as a mediator of cardiovascular disease. Oxidative Medicine and Cellular Longevity 2009, 2 (5): 259–269.
15. Fleming I et al: Endothelum derived hyperpolarizing factor synthase (Cytochrome P4502C9) is a functionally significant source of reactive oxygen species in coronary arteries. Circ Res 2001, 88: 44-51.
16. Gey KF et al: Increased risk of cardiovascular disease at suboptimal plasma concentrations of essential antioxidants: an epidemiological update with special attention to carotene and vitamin C. Am. J. Clin. Nutr. 1993, 57 (suppl.): 787S–797S.
17. Glass CK et al: Atherosclerosis: The Road Ahead. Cell 2001, 104: 503–516.
18. Griendling KK et al: NAD(P)H oxidase: role in cardiovascular biology and disease. Circ Res 2000, 86: 494-501.
19. Guzik TJ et al: Coronary Artery Superoxide Production and Nox Isoform Expression in Human Coronary Artery Disease. ArteriosclerThrombVasc Biol. 2006, 26:333–339.
20. Halliwell B: Biochemistry of oxidative stress. BiochemSoc Trans 2007, 35: 1147-50.
21. Hansson G et al: The role of the lymphocyte. In: Fuster V, Ross R,Topol E, eds. Atherosclerosis and Coronary Artery Disease. New York,NY: Lippincott-Raven 1996, 557–568.
22. Hansson GK et al: The immune system in atherosclerosis. Nat Immunol 2011, 12: 204-212.
23. Harrison D et al: Role of oxidative stress in atherosclerosis. Am J Cardiol 2003, 91:7A -11A.
24. Harrison DG: Cellular and molecular mechanisms of endothelial cell dysfunction. J Clin Invest 1997, 100: 2153-2157.
25. Hirata K et al: Oxidized low density lipoprotein inhibits bradykinin-induced phosphoinositide hydrolysis in cultured bovine aortic endothelial cells. FEBS Lett. 1991, 287:181–184.
26. Holland JA et al: Bradykinin induces superoxide anion release from human endothelial cells. J Cell Physiol 1990, 143: 21-25.
27. Hsies CC et al: Oxidized low density lipoprotein induces apoptosis via generation of reactive oxygen species in vascular smooth muscle cells. Cardiovasc Res 2001, 49: 135-145.
28. Houston M et al: Binding of xanthine oxidase to vascular endothelium. Kinetic characterization and oxidative impairment of nitric oxide-dependent signaling. J Biol Chem. 1999, 274: 4985-4994
29. Hulsmans M et al: Mitochondrial reactive oxygen species and risk of atherosclerosis. Current Atherosclerosis Reports 2012, 14 (3): 264–276.
30. Inoue N et al: Lysophosphatidylcholine inhibits bradykinin-induced phosphoinositide hydrolysis and calcium transients in cultured bovine aortic endothelial cells. Circ Res. 1992, 71:1410–1421.

31. Janssen M et al: Myocardial xanthine oxidoreductase activity in hypertensive and hypercholesterolemic rats. Cardioscience 1993, 4: 25–29.

32. Jialal I et al: Beta-Carotene inhibits the oxidative modification of low-density lipoprotein. Biochim Biophys Acta 1991, 1086 (1): 134-138.
33. Kerr S et al: Superoxide anion production is increased in a model of genetic hypertension: role of the endothelium. Hypertension 1999, 33: 1353- 1358.
34. Landmesser U et al: Vascular oxidative stress and endothelial dysfunction in patients with chronic heart failure: role of xanthine-oxidase and extracellular superoxide dismutase. Circulation. 2002, 106: 3073-3078.

35. Lee RT et al: Mechanical strain induces specific changes in the synthesis and organization of proteoglycans by vascularsmooth muscle cells. *J Biol Chem*. 2001, 276: 13847–13851.
36. Leonarduzzi G et al: Inflammation- related gene expression by lipid oxidation-derived products inthe progression of atherosclerosis. Free RadicBiol Med 2012, 52: 19-34.
37. Libby P: Current concepts of the pathogenesis of the acute coronary syndromes. Circulation 2001, 104: 365–372.
38. Libby P et al: Macrophages and atherosclerotic plaque stability. CurrOpinLipidol. 1996, 7: 330–335.
39. Linster CL et al: Vitamin C: Biosynthesis, recycling and degradation in mammals. FEBS J 2007, 274 (1): 1-22.
40. Lusis AJ: Atherosclerosis. Nature 2000, 407(6801): 233-241.
41. Martinez-Cayuela M: Oxygen free radicals and human disease. Biochimie 1995, 77(3): 147–61.
42. Mehrabian M, Allayee H, Wong J et al: Identification of 5- lipoxygenase as a major gene contributing to atherosclerosis susceptibility in mice. Circ Res 2002; 91: 120–126.

43. Mehrabian M et al: Identification of 5- lipoxygenase as a major gene contributing to atherosclerosis susceptibility in mice. Circ Res 2002, 91: 120–126.

44. Michel T et al: Nitric oxide synthases: which, where, how, and why? J Clin Invest 1997, 100: 2146–2152.

45. Miwa Y et al: Lysophosphatidylcholine inhibits receptor-mediated Ca_2 mobilization in intact endothelial cells of rabbit aorta. ArteriosclerThrombVasc Biol. 1997, 17:1561–1567.

46. Mohazzab-H KM et al: Sites of superoxide anion production detected by lucigenin in calf pulmonary artery smooth muscle. Am J Physiol. 1994, 267: L815–L822.

47. Mohazzab-H KM et al: Lactate and PO_2 modulate superoxide anion production in bovine cardiac myocytes: potential role of NADH oxidase. Circulation 1997, 96: 614–620.
48. Nagel T et al: Shear stress selectively upregulates intercellular adhesion molecule-1 expression in cultured human vascular endothelial cells. J Clin Invest. 1994, 94: 885–891.
49. Pagano PJ et al: An NADPH oxidase superoxide-generating system in the rabbit aorta. Am J Physiol. 1995, 268: H2274–H2280.
50. Paigen B et al: Variation in susceptibility to atherosclerosis among inbred strains of mice. Atherosclerosis 1985, 57: 65–73.
51. Phan SH et al: Xanthine oxidase activity in rat pulmonary artery endothelial cells and its alteration by activated neutrophils. Am J Pathol 1989, 134: 1201-1211.
52. Povoa, HJr et al: Xanthine oxidase and triglycerides in serum of patients with hyperlipoproteinemia, type IV. Biomed. Biochim. Acta 1984, 43:1201–1203.
53. Rajagopalan S et al: Angiotensin II mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation: contribution to alterations of vasomotor tone. J Clin Invest 1996, 97: 1916–1923.
54. Rao GN et al: Active oxygen species stimulate vascular smooth muscle cell growth and proto-oncogene expression. Circ Res 1992, 70: 593-599.
55. Sanders SP et al: Hyperoxic sheep pulmonary Microvascular endothelial cells generate free radicals via mitochondrial electron transport. J Clin Invest 1993, 91: 46-52.
56. Schnabel R et al: Oxdative stress in cardiovascular disease: successful translation from bench to bedside. Circulation 2007. 116: 1338-1340.
57. Sorescu D et al: Superoxide Production and Expression of NOX Family Proteins in Human Atherosclerosis. Circulation. 2002, 105:1429–1435.
58. Spiekermann S et al: Electron spin resonance characterization of vascular xanthine and NAD(P)H oxidase activity in patients with coronary artery disease: relation to endothelium-dependent vasodilation. Circulation. 2003, 107: 1383- 1389.
59. Steinberg D et al: Beyond cholesterol, modifications of low-density lipoprotein that increase its atherogenicity. N Engl J Med 1989, 320: 915–924.
60. Steinberg D: The LDL modification hypothesis of atherogenesis: an update. J Lipid Res 2009, 50(Suppl): S376-S381.
61. Stocker R et al: Role of oxidative modifications in atherosclerosis. Physiol. Rev. 2004, 84:1381–1478.

62. Tardif JC: Oxidative stress and coronary heart disease. Cardiology Rounds 2003, 7.
63. Touyz RM et al: Ang 11-stimulated superoxide production is mediated via phospholipase D in human vascular smooth muscle cells. Hypertension 1999, 34: 976-982.
64. Tuehr DJ: Mammalian nitric oxide synthases. Biochim Biophys Acta 1999, 1411: 217–230.
65. Vasquez VJ et al: Superoxide generation by endothelial nitric oxide synthase: the influence of cofactor. Pros NatlAcadSci (USA) 1998, 95: 9220-9225.
66. Wu D et al: Effect of vitamin E on human aortic endothelial cell production of chemokines and adhesion to monocytes. Atherosclerosis 1999, 147: 297–307.
67. Xia Y et al: Superoxide generation from endothelial nitric oxide synthase. A Ca^{2+}/calmodulin-dependant and tetrahydrobiopterin regulatory process. J BiolChem 1998, 273: 25804-25808.
68. Zafari AM et al: Role of NADH/NADPH oxidase-derived H_2O_2 in angiotensin 11-induced vascular hypertrophy. Hypertension 1998, 32: 488-495.
69. Zalba G et al: Oxidative stress in arterial hypertension: role of NAD(P)H oxidase. Hypertension 2001, 38: 1395-1399.

Figure legends

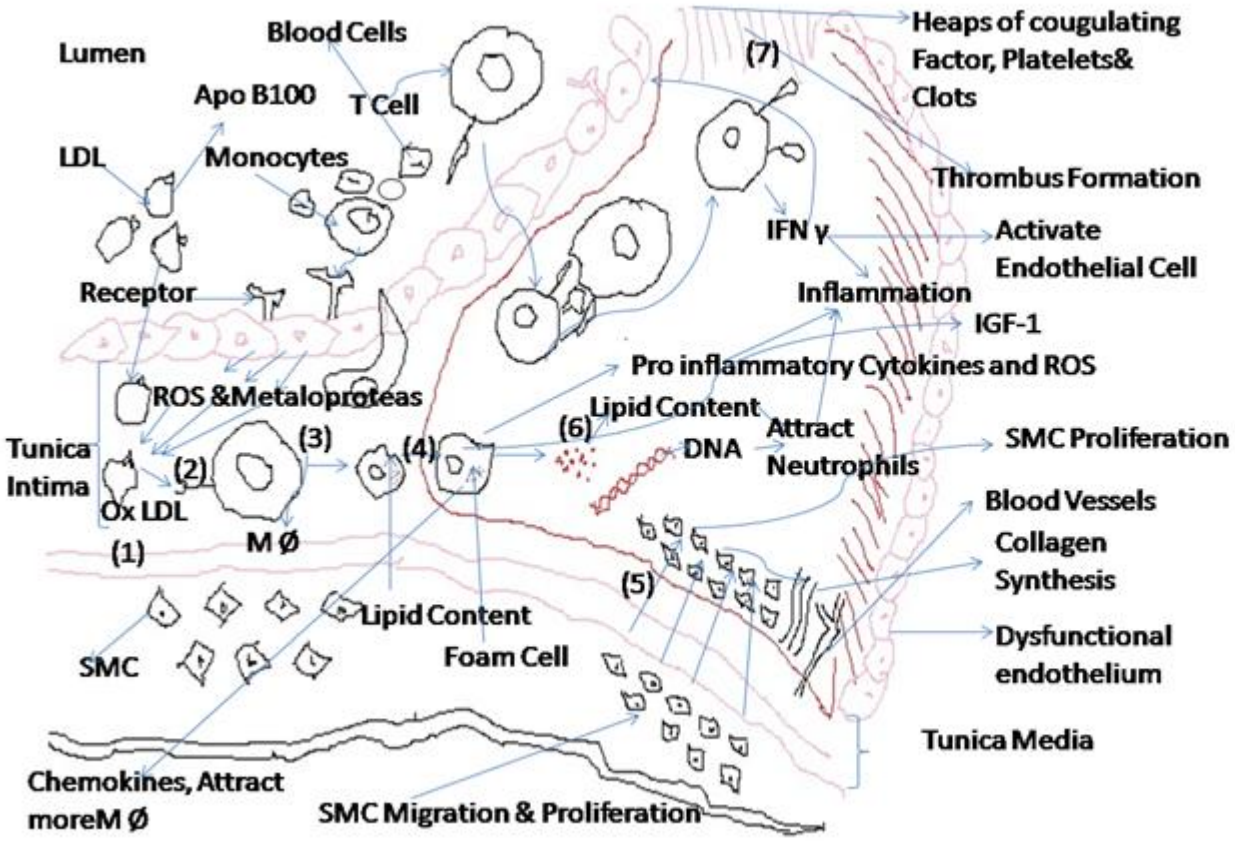

Fig 1. Mechanism of plaque formation in atherosclerosis:

(1) Increased LDL deposit in tunica intima and become oxidised, activate endothelial cells. (2) Adhesion of blood leukocytes to activate endothelium, move to tunica intima. (3) Macrophages take in oxidized LDL and become foam cells. (4) Foam cells promote migration of smooth muscle cells (SMC) from tunica media to tunica intima and SMC proliferation. (5) Increased SMC proliferation, heightened synthesis of collagen. (6) Foam cells dies, lipid content released. (7) Thrombosis → plaque ruptures → blood coagulation → Thrombus → Impedes blood flow.

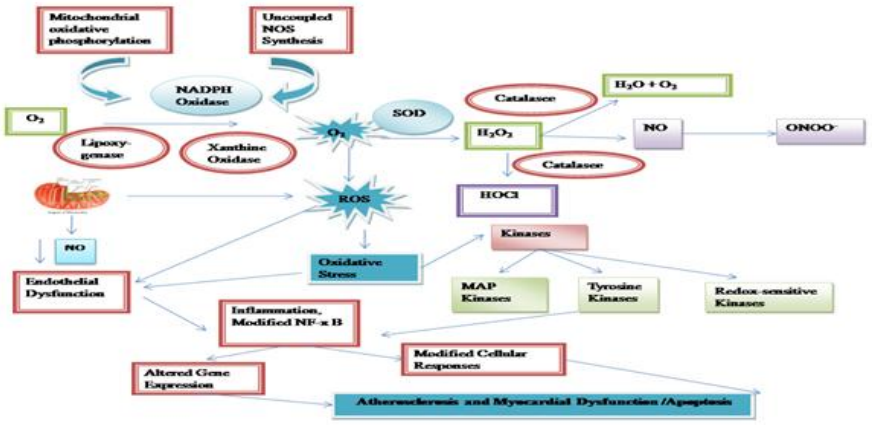

Fig 2. Generation of ROS & RNS in atherosclerosis

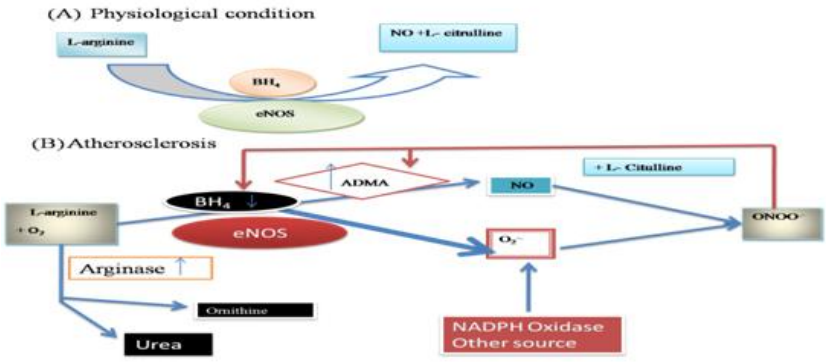

Fig 3. Mechanism of eNOS uncoupling in atherosclerotic endothelial dysfunction: (A) In the presence of co-factor (Tetrahydrobiopterin, BH4) NO from L-arginine produce by endothelial cells under physiological conditions. (B) Arginase activity with the metabolism of L-arginine to urea and ornithine, production of endothelial BH4 decreases and formation of endogenous eNOS inhibitor, asymmetric dimethylarginine (ADMA) increases causes eNOS uncoupling react with NO to produce peroxynitrite ($ONOO^-$) which inactivate BH_4 and increased the accumulation of ADMA in endothelial cells, leading to endothelial dysfunction which

may promote atherogenesis under pathological condition

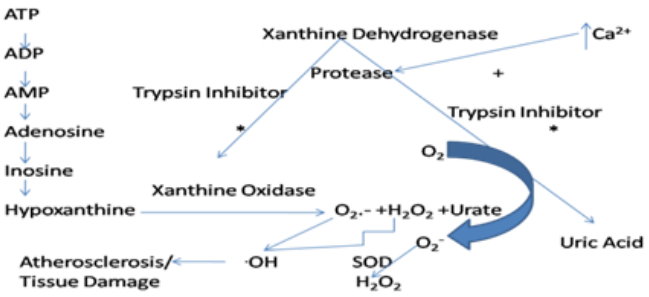

Fig 4. **Mechanism of ROS production through xanthine oxidase:** Xanthine oxidase produce reactive oxygen species and causes tissue injury linked with atherosclerotic lesions.

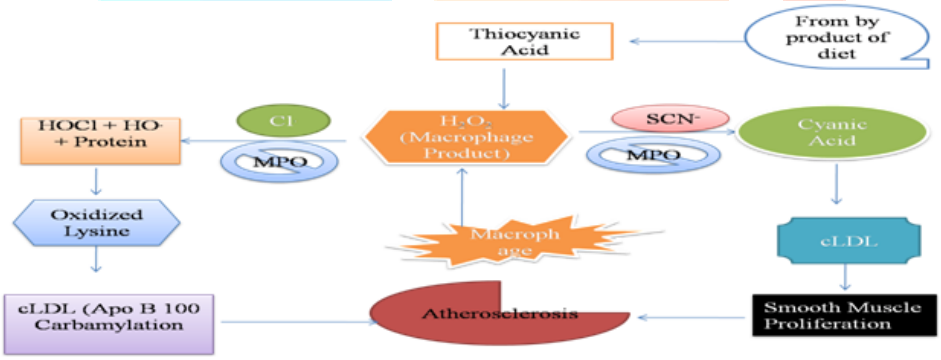

Fig 5. **Mechanism of H_2O_2 production through myeloperoxidase**: Myeloperoxidase produces HOCl from Hydrogen peroxide and chloride ions during LDLcarbamylation, represents a potential molecular pathway that links to diet, smooth muscle cell proliferation and finally lead to atherosclerosis.